



Clinico-Biochemical Diagnosis of Pregnancy Toxemia in Ewes with Special Reference to Novel Biomarkers

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ABSTRACT

Key words:

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biomarkers.

Fifty five ewes aged from 3-5 years were included in this study; these animals were selected from sheep reared in different villages in Menofia and Behera governorates, Egypt. All animals were subjected to careful clinical and laboratory investigations. Accordingly, the selected animals were divided into three groups. Group (A) consisted of 20 ewes showed signs of pregnancy toxemia. Group (B) consisted of 20 heavily pregnant ewes in two or more fetuses determined by ultrasonography. Group (C) consisted of 15 non pregnant ewes. Clinical examination of ewes with pregnancy toxemia reveals anorexia, loss of condition, nervous movement of the eye which is bulging and roaring, grinding of the teeth, champing of the jaw with salivation, spasms in the neck and back muscles and lateral recumbency, odor of acetone in the breath, drowsiness and stiffness of the body. Biochemical findings reveals significance decrease of glucose, cholesterol, total protein, albumin, globulin, T₃, T₄, calcium, sodium and potassium in group A (pregnancy toxemia) when compared to groups B (heavily pregnant) & C (non-pregnant), also significance increase of triglyceride, non-esterified fatty acids (NEFA), β -Hydroxybutyrate, cortisol, AST, ALT, GGT, LDH, urea and creatinine when compared to groups B & C ewes. Further, ewes displaying symptoms of pregnancy toxemia (group A) had a significance increase in serum haptoglobin (Hp), amyloid A and C-reactive protein markers compared to heavily pregnant and non-pregnant ewes.

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1. INTRODUCTION

Ovine pregnancy toxemia (OPT) also known as ketosis is a common metabolic disorder of undernourished ewes due to increased foetal energy requirements in late pregnancy. It is most prevalent in pregnant ewes in twins or triple or in obese ewes (Sargison et al., 1994).

Pregnancy Toxemia is caused by an alteration in carbohydrate utilization in the animal. As a ewe's pregnancy advances, her body needs more energy, at this moment, the size of her rumen declines since the growth of the offspring take up more space leaving less space for the rumen. This leads to the ewe not receiving enough carbohydrates (energy) through her food. So she go to breaking down her own body tissues (usually fat) in order to provide energy for her growing fetuses, thus releasing ketones (a toxic byproduct of fat breakdown) into her blood stream. When this occurs too rapidly, the ewe's body cannot clear the toxic effect of ketones and ketosis or pregnancy toxemia results (Rook, 2000; Van Saun,

2000). Ketosis can occur in obese ewe since fat occupy more space in abdomen resulting a less space for the rumen to hold feed. Additionally, conditions that interrupt feed intake, such as storms, overcrowding, other diseases, can also induce this metabolic disease (Cal-Pereyra et al., 2012). The disease has a significant economic impact on sheep and goat enterprise which reflects directly in national income due to loss of sheep and fetuses (Lima et al., 2012).

The clinical findings in ewes suffering from pregnancy toxemia shows depression, restlessness, grinding in the teeth, sometimes constipation, loss of condition, acetone smell from the mouth, and dystocia. Neurologic signs include blindness, stiffness, incoordination, tremors in neck muscle, convulsions and lateral recumbency (Pough, 2002).

The biochemical findings in pregnancy toxic ewes are characterized by hypoglycaemia and hyperketonaemia resulting in the inability of the animal to maintain its energy requirement (Al-Qudah,

2011). The liver is important for the blood glucose metabolism, for the glucose's tissue supply and because it is virtually the only organ where the gluconeogenesis takes place, so it is good to measure liver function tests although there are small contributions from the kidney (Jyothi et al., 2014; Harmeyer and Schlumbohm, 2006).

Acute phase proteins (APPs) have been used as sensitive, accurate and rapid predictors of inflammatory changes in ruminants (González et al., 2011). However, the APPs in small ruminants are poorly described. The different APPs may play a similar role both in sheep and goat but some differences have been reported (González et al., 2008; El-Deeb, 2012). Haptoglobin (Hp), Serum Amyloid A (SAA) and C-reactive protein are considered as major APPs in both ovine and caprine (Murata et al., 2004). Animal APPs are not only useful for monitoring inflammatory processes for diagnostic and prognostic purposes but also for analyzing various non-inflammatory conditions such as pregnancy, parturition, metabolic diseases and stress, which have previously been considered not to affect APP values (Kent, 1992). C-reactive protein (CRP) plays important roles in protection against infection, clearance of damaged tissue, prevention of autoimmunization and regulation of the inflammatory response (Mold et al., 2002).

The aim of this study was to evaluate the clinical findings, biochemical changes and recent biomarkers in pregnancy toxemic ewes in comparison with heavily pregnant and non-pregnant ewes.

2. MATERIAL and METHODS:

2.1. Animals:

Fifty five ewes aged (3-5) year were included in this study. These animals were selected from sheep reared in different villages in, Menofia and Behera governorates, Egypt. All the selected animals have been reared similarly under unorganized farming with unsatisfactory standards of animal management and feeding.

All animals were subjected to careful clinical and laboratory investigations. Accordingly, the selected animals were divided into three groups. The first group (A) consisted of 20 ewes showed signs of pregnancy toxemia. The second one (B) consisted of 20 pregnant ewes in two or more lambs in the last month of pregnancy determined by ultrasonography. The third group (C) is consisted of 15 non-pregnant ewes.

Periodic clinical and laboratory examinations were applied. Determination of the body temperature, pulse and respiratory rates as well as examination of mucous membranes, lymph nodes and abdomen was conducted according to (Kelly, 1984).

2.2. Sampling:

From each ewe one blood samples were collected by the jugular vein puncture in a tube without anticoagulant for subsequent serum collection and analysis.

2.1.a. Biochemical analysis:

Glucose, Cholesterol, Triglyceride, Non esterified fatty acids NEFA (mmol/l), β -Hydroxybutyrate (mmol/l), Total protein, albumin, Cortisol, T3, T4, alanine transferase (ALT), aspartate transferase (AST), γ -glutamyl transferase (GGT), Alkaline phosphatase (AP), Lactate dehydrogenase (LDH), urea, creatinine, Calcium, Phosphorus, Sodium and potassium; all previous parameters were determined by spectrophotometric method using commercially available test kits supplied by Biomed diagnostics (Germany) and following the manufacturer's instructions. Globulin was determined by the differences between total protein and albumin.

2.1.b. Analysis of novel biomarkers.

Serum Hp, amyloid A and C-reactive protein measured with a commercially available ELISA kit (Phase Hp, Phase SAA and phase C-reactive protein kit, Tridelta Ltd., Ireland), according to the manufacturer's instructions, determined according to the method described by (Becker et al., 1984). The plates were read at 450 nm on a computerized automated micro plate ELISA reader (Bio TEC, ELX800G, USA). All measurements were made in duplicate.

2.1.c. Statistical analysis

The data were analyzed by using one-way analysis of variance (ANOVA). The means in different periods of experiment stage were compared to the control using one way ANOVA followed by Holm Sidak as a post hoc test. All data were analyzed by using Sigma Stat 3.1, statistical software for data analysis (SPSS Inc., Chicago, IL, USA). Values were represented by means \pm standard error (SE). All differences were considered statistically significant at $P < 0.05$ according to (Norman and Baily, 1997).

3. RESULTS:

3.1. Clinical findings:

Ewes with pregnancy toxemia showed anorexia, loss of condition, nervous movement of the eye which is bulging and roaring, grinding of the teeth,

champing of the jaw with salivation, spasms in the neck and back muscles and lateral recumbency, odor of acetone in the breath, drowsiness and stiffness of the body (Fig. 1)

3.2. Biochemical findings

The mean values of the ewes with pregnancy toxemia in comparison with heavily pregnant and non-pregnant ewes are presented in (Table 1). Which show a great significance decrease of glucose, cholesterol, total protein, albumin and globulin in group A (pregnancy toxemia) rather than group C (non-pregnant ewes), also there is mild significant decrease of glucose, cholesterol, total protein, albumin and globulin in group B (heavily pregnant) rather than group C. Also there are a great significance increase of triglyceride, non-esterified fatty acids (NEFA) and β -Hydroxybutyrate in group A (pregnancy toxemia) rather than group C, also there is mild significant increase of triglyceride, non-esterified fatty acids (NEFA) and β -Hydroxybutyrate in group B (heavily pregnant) rather

than group C. Furthermore, there is great decrease in calcium, sodium and potassium activities were determined in the blood of ewes in group A when compared to groups B and C. The opposite trend that there are no changes in phosphorus within groups.

There is a great significance increase of cortisol in group A rather than groups B and C while there are a great significance decrease of T_3 & T_4 in group A rather than groups B and C. (Table 2).

In additions, there are great variations in ALT, AST, GGT, LDH, urea and creatinine activities were determined in the blood of ewes in group A when compared to groups B and C. The opposite trend that there is no changes in AP within groups (Table 3).

3.3. The recent biomarker:

There are a great significance increase of serum Hp, amyloid A and C-reactive protein in group A (pregnancy toxemia) rather than group C (non-pregnant), while group B show mild significance increase of serum Hp, amyloid A and C-reactive protein when compared with group C. (Table 4).

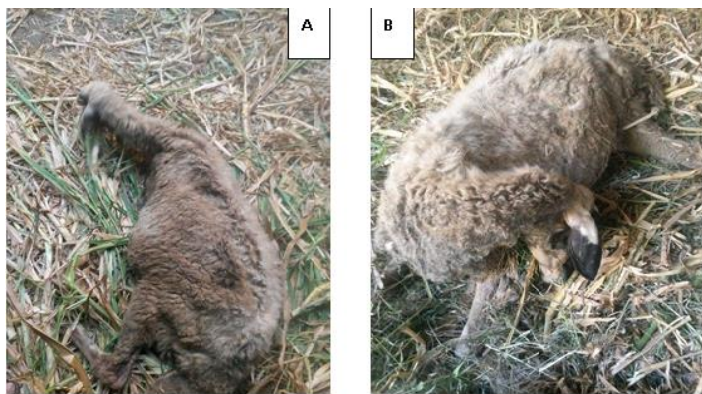


Fig. 1: (A) Spasm in neck muscle with distended abdomen. (B) Shows stiffness in the whole body with directed head to the flank region.

Table (1):- Biochemical parameters in pregnancy toxemic ewes compared with heavily pregnant and non-pregnant ewes.

Parameters	Group A Mean \pm SE	Group B Mean \pm SE	Group C Mean \pm SE
Glucose (mg/dl)	36.33 \pm 0.15 ^c	61.15 \pm 0.15 ^b	72.65 \pm 0.17 ^a
Cholesterol (mg/dl)	48.45 \pm 0.15 ^c	69.05 \pm 0.17 ^b	79.33 \pm 0.21 ^a
Triglyceride (mg/dl)	110.45 \pm 0.034 ^a	86.22 \pm 0.034 ^b	58.31 \pm 0.12 ^c
NEFA (mmol/l)	1.81 \pm 0.04 ^a	1.23 \pm 0.02 ^b	0.44 \pm 0.01 ^c
β -Hydroxybutyrate (mmol/l)	7.81 \pm 0.05 ^a	1.23 \pm 0.03 ^b	0.81 \pm 0.01 ^c
Total proteins (mg/dl)	4.05 \pm 0.15 ^c	6.01 \pm 0.17 ^b	7.25 \pm 0.21 ^a
Albumin (mg/dl)	1.73 \pm 0.13 ^c	2.32 \pm 0.13 ^b	3.11 \pm 0.21 ^a
Globulin (g/L)	2.32 \pm 0.17 ^c	3.69 \pm 0.07 ^b	4.14 \pm 0.11 ^a
Calcium (mmol/l)	1.87 \pm 0.32 ^c	2.77 \pm 0.41 ^b	2.98 \pm 0.14 ^a
Phosphorus (mmol/l)	1.96 \pm 0.02 ^a	2.0 \pm 0.1 ^a	2.21 \pm 0.01 ^a
Sodium (mEq/l)	104.12 \pm 0.28 ^c	165.33 \pm 0.06 ^b	178.02 \pm 0.42 ^a
Potassium (mEq/l)	3.1 \pm 0.06 ^c	4.7 \pm 0.04 ^b	5.4 \pm 0.07 ^a

Means within the same row having the different letters are significantly different at ($P < 0.05$).

Table (2):- Hormonal parameters in pregnancy toxemic ewes compared with heavily pregnant and non-pregnant ewes.

Parameters	Group A Mean \pm SE	Group B Mean \pm SE	Group C Mean \pm SE
cortisol (nmol/l)	1056.34 \pm 68.94 ^b	698.95 \pm 0.15 ^{ab}	523.05 \pm 53.05 ^a
T3(nmol/l)	12.22 \pm 1.54 ^a	15.05 \pm 0.17 ^{ab}	17.11 \pm 5.01 ^b
T4 (ng/ml)	7.22 \pm 0.94 ^a	9.22 \pm 0.34 ^{ab}	11.25 \pm 1.33 ^b

Means within the same row having the different letters are significantly different at (P<0.05).

Table (3):- Liver and kidney function tests in ewes with pregnancy toxemia compared with heavily pregnant and non-pregnant ewes.

Parameters	Group A Mean \pm SE	Group B Mean \pm SE	Group C Mean \pm SE
AST (u/l)	166.20 \pm 0.48 ^a	67.20 \pm 0.15 ^{ab}	55.20 \pm 0.50 ^b
ALT(u/l)	87.80 \pm 0.53 ^a	39.60 \pm 0.81 ^{ab}	25.70 \pm 0.68 ^b
GGT(u/l)	78.80 \pm 0.40 ^a	55.90 \pm 0.63 ^{ab}	44.50 \pm 0.03 ^b
AP(u/l)	90.40 \pm 0.92 ^a	89.50 \pm 0.29 ^a	88.00 \pm 0.52 ^a
LDH (u/l)	498.10 \pm 0.07 ^a	422.10 \pm 0.08 ^b	401.10 \pm 0.71 ^c
Urea(mg/dl)	47.93 \pm 0.09 ^a	29.93 \pm 0.22 ^{ab}	27.03 \pm 0.01 ^b
Creatinine (μ mol/L)	102.02 \pm 0.28 ^a	80.19 \pm 0.11 ^{ab}	72.20 \pm 0.71 ^b

Means within the same row having the different letters are significantly different at (P<0.05).

Table (4): Novel biomarkers in ewes with pregnancy toxemia compared with heavily pregnant and non-pregnant ewes.

Parameters	Group A Mean \pm SE	Group B Mean \pm SE	Group C Mean \pm SE
Haptoglobin (mg/l)	1.41 \pm 0.04 ^a	0.06 \pm 0.02 ^{ab}	0.05 \pm 0.001 ^b
Serum amyloid A (mg/l)	30.26 \pm 0.57 ^a	6.36 \pm 1.6 ^{ab}	5.24 \pm 0.53 ^b
C-reactive protein (μ g/mL)	199.35 \pm 1.01 ^a	112.88 \pm 0.41 ^{ab}	84.88 \pm 0.61 ^b

Means within the same row having the different letters are significantly different at (P<0.05).

4. DISCUSSION

The last two months of gestation in pregnant ewes are a critical period because most of the foetal growth occurs during this period. Pregnancy toxemia in small ruminants occurs because of the competition for glucose between the pregnant ewes and its fetuses (Bergman, 1993; Bulgin, 2005; Duehlmeier, et al., 2013).

Ewes with pregnancy toxemia shows anorexia, loss of condition, nervous movement of the eye which is bulging and roaring, grinding of the teeth, champing of the jaw with salivation, spasms in the neck and back muscles and lateral recumbency, odor of acetone in the breath, drowsiness and stiffness of the body these signs may attributed to ischemia, hypoglycemia and epilepsy that affects energy metabolism by arresting cellular energy production or pathologically enhancing energy consumption (Auer and Siesjo, 1988). The present findings are in agreement with (Scott et al., 1998; Henze et al., 1994). The authors found that plasma glucose concentrations of pregnancy toxemic ewes were significantly lower compared to anorexic and healthy ewes at the same stage of gestation.

The great decrease of glucose agreed by (Marteniuk and Herdt, 1988) and this attributed to the animal cannot meet the glucose demands of the fetal placental unit and hypoglycemia develops. And the elevation of β -hydroxybutyrate resulted in a significant drop of glucose turnover (Schlumbohm and Harmeyer, 2004) and real cause of the hypoglycemic effect and high concentration of β -hydroxybutyrate is decline of food intake and glucose turnover (Tharwat and Al-Sobayil, 2014).

The marked increase in serum triglyceride and NEFA may be attributed to increase of fat break down and mobilization from fat stores to face marked decrease of glucose. (Marteniuk and Herdt, 1988) and the decrease of cholesterol may attributed to decrease food intake and hepatic insufficiency (El-Sebaie, 1995). Furthermore there is a great drop in total protein, albumin and globulin in pregnancy toxemic ewes in comparison other groups and this results attributed to increased protein catabolism, decomposing fetuses or terminal kidney failure and this agreed by (Andrews, 1997).

Ewe that in pregnancy toxemia or carrying 2 or 3 fetuses and with a marked negative energy balance

has all its gluconeogenic mechanisms working very efficiently which lead to that all glucocorticoid blood concentrations are elevated in ovine ketosis (Bergman, 1993). Prolonged hypoglycaemia also lead to hyperactivity of the adrenal glands, with increased cortisol secretion, these explain the higher cortisol level. Cortisol increased due to reduced hepatic metabolism as well as the hypoglycemia and continuing stress levels. The significant decrease in T3 and T4 in pregnancy toxemic ewes may be caused by excessive secretion of cortisol as there is a negative correlation between free T4 and cortisol as explained in (Hackney and Dobridge, 2009) the effect of starvation lead to hormonal signals which initiate energy preservation. Insulin, T4, and T3 are important hormones in the regulation of energy homeostasis. The decreases in T4 in pregnancy toxemic ewes in the present study were similar to that noticed in ewes (Kulcsár et al., 2006) Goats (Hefnawy et al., 2011) and ferret (Prohaczik et al., 2009) with pregnancy toxemia. And the significance decreases in T3 is disagreed with (Hefnawy et al., 2011).

The higher levels of AST, ALT, GGT and LDH activities in the pregnancy toxemic ewes, may attributed to liver damage (Kaneko et al., 1997). These results are agreed with those noticed by (Peneva and Goranov 1984; El-Deeb, 2012) they said that, the levels of AST, ALT, GGT and LDH activities were significantly higher and correlated positively with the rise of ketonemia and ketonuria in cows with subclinical ketosis, also there is no changes in AP between the groups. There is great increase in urea and creatinine and this caused by increased protein catabolism, by decomposing fetuses or by terminal kidney failure (uraemia) and this agreed by (Andrews, 1997).

There are a great decrease in the serum levels of calcium, sodium and potassium in diseased ewes and this attributed to there were disturbances in the electrolytes and some minerals which may be attributed to stress of starvation, dehydration, electrolytes imbalance and role of the kidney in the pathogenesis of pregnancy toxemia or also due to enhanced lipolysis that can induce hypocalcemia (Jopp and Quinlivan, 1981; Judith and Thomas, 1988) found that hypokalemia and hypocalcaemia that are associated with pregnancy toxemic ewes may be attributed to anorexia and metabolic acidosis (Rook, 2000) or inadequate feed intake and incomplete renotubular absorption of potassium (Henz et al., 1998). Also the high demand of offspring to

calcium at the late stage of gestation leads to significant decrease of calcium, decrease Ca level is also due to high circulating cortisol levels and the fatty liver interfering with hydroxylation of vit. D (Andrews, 1997). Also there is no change in the level of phosphorus.

After determination of the recent biomarker we found great increase in the levels of Hp and SAA in pregnancy toxemic ewes when compared to other groups. These results are in agreement with findings of (Ametaj, 2005; Murphy et al., 2009; El-Deeb, 2012) which found a great relation between Hp and lipid metabolism, also Hp has significance increase with starvation in cows. In ruminant there is a relationship between APPs and lipid mobilization (González et al., 2011). The significant increase in APPs found in ewes with pregnancy toxemia could be related to the changes in lipid metabolism that occur in this process (Scott et al., 1998). Also (Yoshino et al., 1993) document the relationships between inflammatory mediators and metabolic disorders, also detected Hp in serum of cows with hepatic lipidosis (fatty liver). There is great increase in the levels of C-reactive protein in pregnancy toxemic ewes when compared to other groups, these attributed to the relation of health status of the animal with C - reactive protein (Conrad and Benyo, 1997).

5. CONCLUSION

Pregnancy toxemia affects some ewes at advanced stages of pregnancy due to twinning or obesity which cause restricting rumen capacity, that leading to decrease blood glucose level with subsequent lipid mobilization for providing body energy, followed by appearance of nervous manifestation on the ewes and changes in blood profile including glucose, protein, fat metabolites, some hormonal and elements. Inflammatory mediators (biomarkers) including serum haptoglobin, amyloid A and C- reactive protein have a great relation with pregnancy toxemia and health status of the ewes and their increase reflect the metabolic disorders and lipid mobilization, so APPs could be used as additional diagnostic biomarkers for pregnancy toxemia in ewes. So must avoid any starvation and negative energy balance especially in advanced stages of pregnancy in ewes to avoid significant economic losses.

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